The Structure and Life-History of Crithidia melophagia (Flu), an Endo-parasite of the Sheep-Ked, Melophagus ovinus.

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With Plates 12 and 13 and 15 Text-figures.

Contents.

Introduction.

The part played by insects as agents in the transmission of the pathogenic organisms of sleeping sickness and other protozoal diseases gives great importance to the investigation of the parasites found within them. It is necessary for anyone seeking developmental stages of pathogenic flagellate Protozoa to have also a first-hand working knowledge of the possible flagellates that may be purely parasites of the insect involved, for certain stages of insect flagellates may resemble possible developmental phases of such organisms as Trypanosomes. Much useful information regarding stages of flagellates can be gained from the study of such a parasite as Crithidia melophagia (Flu), occurring in the alimentary tract, ovaries, and ova of the sheep-"ked," Melophagus ovinus. This insect, which is blood-sucking, is also known as the sheep-"tick" or sheep-"louse." It belongs really to the Diptera (Hippoboscidæ), possessing extremely reduced wings.

Crithidia melophagia (Flu) was recorded by E. Pfeiffer in 1905, but not named by him. The parasite is of peculiar interest, for I am able to bring forward evidence of a double mode of infection, both hereditary and casual. Swingle (1909) studied the flagellate stages and briefly described infection in the egg of Melophagus. Flu (1908) found parasites in the gut, ovaries, and larva, but was not clear as to the mode of infection (see p. 211).

Owing to conditions of environment it was impossible to conduct the whole of this investigation in a large city. Consequently the work has entailed travelling, and I have to thank many friends in agricultural centres for their kindly help.

Material and Methods.

Many specimens of Melophagus ovinus were examined during a long period of investigation, but owing to the effective operation of the dip laws in England there was

much difficulty in obtaining the "keds."[1] Indeed, it seems probable that the sheep-ked may soon become almost extinct in England. Those obtained came in very small numbers from many localities in the south of England, namely, Sussex, Hampshire, Kent, Middlesex, and Gloucestershire. I also received a number of keds from different parts of Scotland, but these never contained the Crithidia.

Many of the Melophagus, however, were infected by a fungus (see Appendix II). Where fungus was present Crithidia very rarely occurred. I shall show later, from experimental evidence, that this fungus was fatal to the Crithidia (p. 210).

Unlike Swingle (1909), who found that practically every Melophagus he examined in Nebraska was infected with Crithidia, I found that this was very far from being the case. Much depended on the locality from which the Melophagus was obtained. The more heavily infected individuals came from the southern districts of England. Often entire stocks of keds from one locality proved to be uninfected. Again, it was impossible to keep keds alive more than three days after their removal from the sheep.

Both young and adult Melophagus and many puparia in all stages of development were carefully examined. Raising puparia naturally upon a sheep was tried, but was not an easy matter, and as one could not be sure of having infected keds, there was always a percentage of uninfected puparia.

For observations of the living organism two methods of procedure were followed. The alimentary canal was isolated and divided into œsophageal, crop, stomach, intestinal and rectal portions, which were separated one from another. These were either teased with needles, mounted in 0·75 per cent. salt solution, and covered, the cover-slip being carefully vaselined, or the contents of the isolated portions of the gut were expelled by gentle pressure, and these only were examined, being mounted as before. Alkaline methylene

[1] In this paper I shall frequently use the term "ked" to denote Melophagus ovinus.

blue and neutral red were occasionally used as intra-vitam stains and were sometimes useful.

For fresh preparations used in work on hereditary infection, the ovaries and gut were dissected out very carefully, kept as far as possible relatively in situ, and mounted in 0·75 per cent. NaCl solution. The behaviour of the Crithidia visible through the walls of the gut and their action when they passed out from it were then most carefully watched.

I have attached very great importance to the study of the living organism in all its phases.

For making permanent preparations the alimentary tract of the Dipteran host was carefully removed and divided into portions as before. These isolated portions were usually teased very finely and fixed wet. Formalin vapour and osmic acid vapour were chiefly used for instantaneous fixation of the hanging-drop preparations, which were then spread. The preparations were subsequently treated with methyl or ethyl alcohol. Corrosive-acetic-alcohol (Schaudinn's fluid) and Bouin's fluid (slightly modified and containing a little alcohol) were also used for fixation.

Various stains were employed. Giemsa's stain gave some pretty results; thionin acted rapidly and well; iron-hæmatoxylin, carefully differentiated with iron-alum, was very serviceable; while gentian violet and Delafield's hæmatoxylin were of great use, particularly in obtaining details of the membrane and flagellum.

In the investigation of Crithidia melophagia, as in all other flagellates on which I have worked, I found that preparations mounted in neutral Canada balsam were superior to dry films or to films mounted in any other media.

Preparations of ovaries, eggs, and puparia were treated similarly. Special methods adopted are detailed in the section dealing with hereditary infection (p. 204).

Distribution of the Parasite in the Host.

The Crithidia parasitic in the alimentary canal of Melophagus are often mixed with the blood obtained by the ked

from the sheep. This blood from the sheep in the œsophagus, crop, and anterior part of the stomach of Melophagus is always fluid, and of an extremely bright red colour. That in the remaining part of the stomach is duller red but fluid, and in the intestine the blood, now semi-digested, is always darker in hue, sometimes brownish or greenish, while in the extreme rectum it is black. The enhanced red colour in the anterior portions of the alimentary canal has been shown experimentally to be associated apparently with the presence of an anti-coagulin in the digestive tract of the sheep-ked (see Appendix III).

Crithidia can be found throughout the length of the alimentary canal of Melophagus ovinus. In the anterior parts of the canal they are small, rounded, non-flagellated forms, which, when they come in contact with the blood, rapidly develop and divide, the products of division becoming the typical flagellates found throughout the rest of the canal. The parasites, after this rapid development, pass backwards towards the partly digested blood, which would appear to be a medium more suited to their requirements. In the posterior third of the stomach there are large numbers of young flagellates which form great aggregation rosettes (Pl. 12, fig. 43) and clumps, while true division rosettes are also present (Pl. 12, fig. 56).

In the intestine the same holds good. When many Crithidia are present in a ked, they usually swarm in the fore-part of the intestine. Repeated division occurs in the intestine, so that small flagellates are found in the rectum. Most of these attach themselves to the gut-wall or to débris and encyst, the resting (post-flagellate) stage of the parasite then being found on the walls of the rectum and in the fæces.

The ovaries and ova serve as places in which a kind of post-flagellate development occurs, the ova being penetrated by flagellate forms of Crithidia, which rapidly lose their flagella and ultimately round themselves off, and pass through a resting stage (Pl. 13, figs. 57–94).

The Malpighian tubules of Melophagus ovinus are sometimes invaded by Crithidia melophagia, but this is not common.

Parasites were more numerous in female than in male keds.

Repeated investigation of sheep's blood failed to show the presence of any flagellate therein. Flu and Swingle obtained similar results. C. melophagia is, then, purely a parasite of Melophagus ovinus.

Movements.

The movements of C. melophagia are very vigorous. The parasites are even more active than C. gerridis (see Porter [1909], p. 352). As in C. gerridis, the membrane takes an important share in locomotion, but the movements of the body of C. melophagia are not so noticeable as in the parasite of the water-bug.

When C. melophagia was examined under the water immersion (2·5 mm.) objective, the movements of the less active organisms could be analysed. In progression the organism moves with its flagellum foremost, and the latter executes vigorous, slightly spiral, boring movements. The body also aids in progression, for waves pass from the posterior end towards the flagellum, causing a series of peristaltic-like swellings. The body of the parasite seems to become shorter during this period, and then by relaxing to move forwards. The bead-like swellings due to undulatory movements are more noticeable in certain areas, and in the living organism myonemes could be sometimes seen both on the body and in the membrane in these regions. Flu has also figured myonemes on some of the parasites he drew, and observation of them in life confirms his work, but it was with the greatest difficulty that I could find myonemes in stained specimens (Pl. 12, figs. 17, 18, 40, 42, 45).

The body of C. melophagia, compared with that of C. gerridis, is relatively rigid, but slight twisting movements do occur. The previous workers on C. melophagia are agreed

as to this rigidity. The anterior end, to which the flagellum and undulating membrane is attached, is naturally more flexible than the posterior end, and its movements are more marked.

Movements of contraction of the posterior end of the body of C. melophagia result in a temporary concentration of the protoplasm around the nucleus of the organism. The body then resembles a short, thick pear, drawn out at its anterior end into a long, narrow stalk. Sometimes the body remains in this condition, which is fairly common in forms about to encyst, and in such forms withdrawal or degeneration of the flagellum, followed by the secretion of a thin gelatinous wall, completes the encystment. In other parasites from the stomach, where no encystment occurs, this concentration of the protoplasm in the nuclear region is not so marked, and when relaxation occurs the organism is propelled forward with a very slight jerk, and repetition of the contraction follows, as has been before described. The jerking is never so marked as in Herpetomonas, for the membrane has the effect of producing smoothness of motion.

Reversal of the direction of motion occurs and is very rapid. The flagellum swings out, describing a semi-circle, of which the body acts as the diameter for an instant, but the force of the movement of the flagellum is so great that the body also swings outwards in a line with the flagellum, and the organism moves away, not exactly in the same course as before, but in one at a very small angle to it. The path of the organism is frequently parabolic in nature.

Many peculiar movements can be observed when C. melophagia is endeavouring to free itself from débris in the lumen of the gut. Much writhing, both of the flagellum and body of such a parasite, is then seen, and the organism often swings round and round, the point of attachment serving as the centre of rotation. If the posterior end should be attached, the flagellum executes violent lashings and spiral movements, these latter not being, as a rule, very noticeable in the normal organism.

Occasionally I have seen the flagellum and membrane of specimens of C. melophagia torn away from the body, and for a few seconds after, the flagellum executed intermittent flickers or lashing movements before it finally became still.

Aggregation-rosettes (Pl. 12, figs. 41, 43; Pl. 13, figs. 95, 96) are common in C. melophagia. Rosettes seem to move fairly as a whole, and I have watched them rotate rather quickly. Each individual of such a rosette is attached by its flagellum to débris, usually epithelial in nature, and moves up and down in a slightly inclined plane.

In division the movements of the daughter organisms are very noticeable. I will defer the description of their motion until division is discussed.

During encystment in the rectum of the host, which occurs with some of the parasites, movement of the nucleus towards the flagellar end of the organism occurred. I have also seen the migration of the nucleus from the mid-region of the body to near the flagellum during periods of violent movement of the latter organella. I have never seen migration of the blepharoplast in living organisms under similar conditions, though it may occur at times, since blepharoplasts can occasionally be found in the post-nuclear region (Pl. 12, figs. 40, 42), as well as by the side of the nucleus (Pl. 12, fig. 33) in different stained specimens. By far the commonest position for the blepharoplast is the pre-nuclear one. The other movements occurring during encystment will be described in the section of the paper dealing with that subject (see p. 200 and text-figures 1–10).

Morphology.

The life-cycle of Crithidia melophagia may be conveniently divided into three stages, which gradually merge into one another. They are—the pre-flagellate, flagellate, and post-flagellate stages. The morphology of these forms may now be described.

The Pre-flagellate Stage.

The early pre-flagellate stages of C. melophagia are more or less oval or rounded bodies (Pl. 12, figs. 1–6), varying from 4·5 μ to 6 μ long, and from 1 μ to 4·5 μ broad. They are most abundant in the fore-gut of young Melophagus, but the pre-flagellate stage is passed through with great rapidity and is easily missed. This probably accounts for the very brief references to these small forms by Flu and Swingle.

The protoplasm of the pre-flagellate forms is very finely granular (Pl. 12, figs. 1–5). The nucleus is usually round and not quite central in position (Pl. 12, figs. 1, 9–12). The bar-like blepharoplast (kinetonucleus) is very deeply staining, and lies either below (Pl. 12, figs. 2, 10) or to one side of the nucleus (Pl. 12, figs. 1, 6). A chromatophile area with its chromatin in a very diffuse condition is sometimes fairly prominent, and from this a fine thread arises, which grows outwards, forming the flagellum (Pl. 12, figs. 9, 10), and appearing to draw out the end of the body with it (Pl. 12, figs. 11–13), while the periplast of the body forms the membrane (Pl. 12, figs. 14–20). The posterior end elongates at the same time (figs. 16–18) and the flagellate form (Pl. 12, figs. 19, 20) is assumed. This development is in accord with that of C. gerridis and C. tabani, and I have watched these processes in living specimens of both C. gerridis and C. melophagia.

Division of pre-flagellate forms can occur before the development of the flagella (Pl. 12, figs. 3, 4). This will be described in the section dealing with division.

The Flagellate Stage.

The mature flagellates vary very much in size, the variation being due to division and growth. Very large forms (Pl. 12, figs. 44, 45) may be as much as 50 μ to 75 μ long, this measurement including the flagellum,[1] while short forms just flagel-

[1] It is almost impossible to differentiate between the limiting areas of the body, the membrane and the free flagellum of C. melophagia, as so much variation occurs in different specimens.

lated (Pl. 12, figs. 18, 19) in the crop, or the small forms produced by division prior to encystment (Pl. 12, figs. 20, 21; 99) are very much smaller (12 μ to 20 μ long). The breadth of the flagellates varies from 1·5 μ to 2·8 μ.

The protoplasm of C. melophagia is very slightly alveolar or almost hyaline, differing therein from the more alveolar protoplasm of C. gerridis. There is no suggestion of large permanent vacuoles or of a cyto-pharynx. Occasionally the protoplasm is more granular at the posterior end (Pl. 12, figs. 30, 34) and slight alveolation occurs there. At the anterior end, near the origin of the flagellum, the remains of the chromatic area, from which the flagellum arose, sometimes persist.

The nucleus (trophonucleus) of C. melophagia is oval (Pl. 12, figs. 21–24) or rounded (figs. 26, 30, 32) and somewhat vesicular. There is a fair amount of chromatin present, which may consist of a number of very fine granules, evenly distributed (fig. 32), or the chromatin may be concentrated into about eight masses (fig. 44), or, as is often the case, the chromatin is present in the form of bars (figs. 25–29), which sometimes extend across the whole breadth of the nucleus (figs. 34–37), less frequently across part of its breadth (figs. 24, 42), or in an even more rare condition dots and bars occur in the nucleus of the same organism (figs. 30, 39). In certain cases the chromatin of the nucleus may be concentrated into a central mass (fig. 23).

The nuclear membrane is fairly distinct in most of the specimens I have examined. I think that such a membrane must be present to keep together the nuclear material during the migrations of the nucleus seen during life.

The blepharoplast (kinetonucleus) of C. melophagia is very evident in a stained preparation, for it colours deeply whatever stain be employed. Like the nucleus, it can also be seen in life as a small bright refractile bar. In some cases it is slightly bowed or curved (Pl. 12, fig. 32), or oval (Pl. 12, fig. 34). It is dumb-bell-shaped in forms about to divide (Pl. 12, fig. 44). The blepharoplast, which is typically

rod-like, usually lies transversely across the organism (Pl. 12, figs. 21–28). It is exceptional to find it in any position other than anterior to the nucleus, though on a few occasions the blepharoplast was at the posterior end of the body (Pl. 12, figs. 40, 42), but in these cases the flagellum originated in a pre-nuclear position.

As a rule the blepharoplast shows no differentiation of structure (Pl. 12, figs. 21–39), but sometimes in dividing forms, in which the blepharoplast is dumb-bell shaped, there seems to be a concentration of chromatin in the ends of the dumb-bell (Pl. 12, figs. 40, 44, 45). A clear area (Pl. 12, fig. 31) is often present around the blepharoplast.

Chromidia are present, scattered in the general protoplasm (Pl. 12, figs. 25, 37, 39). They stain in the same way as the nucleus, and less densely than the blepharoplast. The occurrence of such chromatoid granules at division (Pl. 12, fig. 45) suggests that they have been given off from the nucleus into the general protoplasm, and exercise some controlling influence over the same.

The undulating membrane and the flagellum.—The flagellum originates from a chromatic area in the pre-flagellate form, and is attached to the body by a narrow membrane (Pl. 12, figs. 21–46), which is a periplastic outgrowth of the anterior end of the body. There is but one flagellum in any single, undividing individual (Pl. 12, figs. 21–39). The flagellum is thick, but gets thinner towards its free end (Pl. 12, figs. 40, 45). At times it appears to show very fine transverse striations.

In stained specimens the membrane sometimes shows myonemes (Pl. 12, figs. 39, 42, 45), though, curiously enough, the myonemes were much more obvious in some of the living specimens that I examined. Flu described myonemes in C. melophagia, but figured the myonemes as accompanying a central spindle. This latter feature I have never seen.

A basal granule (blepharoplast of Minchin) is often present (Pl. 12, figs. 17, 27, 33, 42, 45) between the point of origin of the flagellum and the blepharoplast (kinetonucleus).

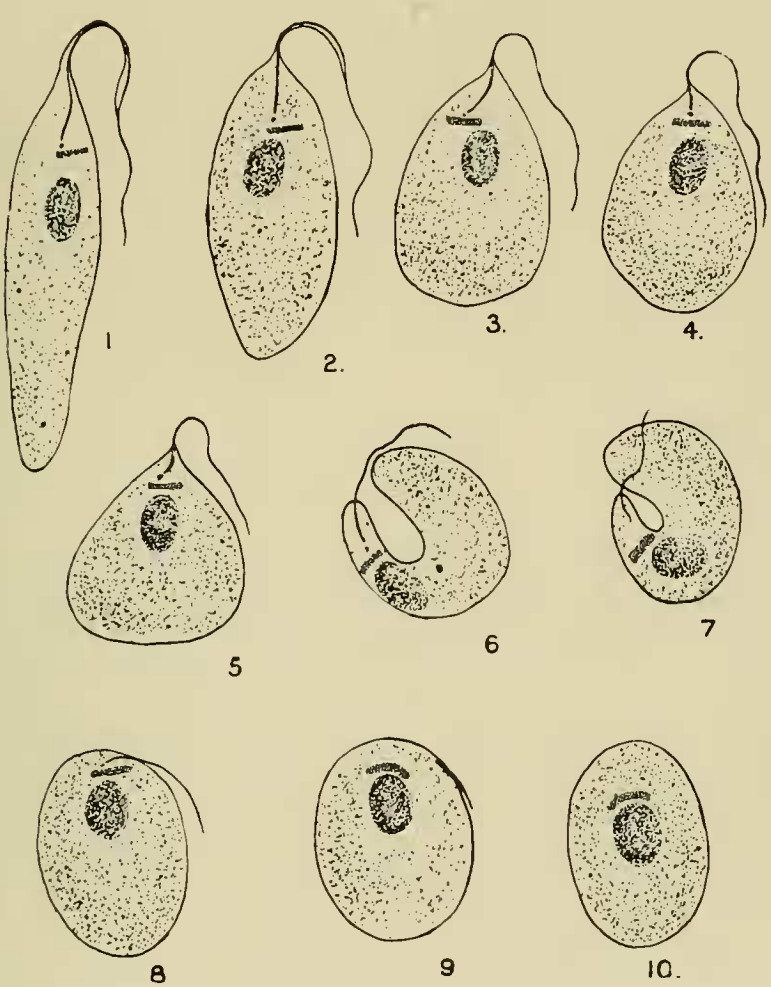
The Post-flagellate Stage of C. melophagia in th Rectum of Melophagus ovinus.

The preparation of Crithidia melophagia for life outside the body of the host occurs in the rectum of the sheep-ked. Large numbers of small flagellates (Pl. 12, figs. 27–29) are present in the hind gut, also some forms in process of division (Pl. 12, figs. 97, 98). The small forms attach themselves to the wall of the rectum and encyst there, but encystment can be watched when the rectal contents are expressed on to a slide and examined under the microscope. The flagellate (text-fig. 1) at first executes violent lashing movements with its flagellum, and during this motion migration of the nucleus nearer the flagellar end of the organism frequently occurs (text-fig. 2). At the same time the body of the Crithidia shortens and thickens (text-figs. 3, 4; Pl. 13, fig. 100), waves of contraction passing rhythmically down the body, which gradually may become somewhat triangular (text-fig. 5; Pl. 13, fig. 101). The flagellum meanwhile shortens (text-figs. 5, 6), and the organism may bend on itself (text-figs. 6, 7) during this period. Concentration of the protoplasm occurs, the flagellum becomes less wavy (text-fig. 7), and, little by little, it contracts nearer the body (text-figs. 8, 9; Pl. 13, figs. 102–106) and is withdrawn, the parasite becoming oval (text-fig. 10; Pl. 13, figs. 109–112). The organism at this time becomes surrounded by a thin layer of refractile, gelatinous substance, which rapidly hardens to form a closely adherent resistant cyst-wall. The oval bodies (Pl. 13, figs. 109–114) so produced are post-flagellate forms, which become detached from the walls of the rectum, and pass out with the fæces of the ked, from which fæces they can be recovered. These cysts, which measure from 2·5 μ to 5·5 μ by 1·5 μ to 3 μ, serve for the infection of other Melophagus ovinus.

All Crithidia melophagia do not go through a post-flagellate stage in the gut of their host. Some, after passing a portion of their existence as flagellates in the gut of the ked,

pierce the walls of the alimentary tract and make their way to the ovaries of the ked, where their development is continued.

TEXT-FIGURES 1–10.



Encystment of Crithidia melophagia in the rectum.

Text-figs. 1–5.—Parasite rounding off and flagellum disappearing.
Text-figs. 6–7.—Show bending of parasite on itself.
Text-figs. 8–10.—Final stages in loss of flagellum and assumption of typical cyst form.

Swingle (1909, p. 104) has described thick-walled cysts. I have but rarely seen the thick-walled forms (Pl. 13, fig. 114), most of the cysts found being thin-walled.

Longitudinal Division.

The longitudinal division of the living organism has been frequently watched. While the movements of the dividing flagellates are noticeable, those of the smaller dividing pre-flagellates are far less marked.

When a flagellate is about to divide, the protoplasm of the posterior end concentrates somewhat in the nuclear region, and the organism appears to shorten. The protoplasm migrates from the centre of the parasite towards the sides, so that a comparatively clear area is left at the centre (Pl. 12, fig. 46). The greatest change at this stage is seen in the blepharoplast and flagellum. The blepharoplast becomes slightly dumb-bell-shaped (Pl. 12, figs. 44, 45) and gradually constricts into two (Pl. 12, fig. 46). The flagellum splits rapidly at the body end (Pl. 12, fig. 46), and then, more slowly, the halves become free. The nucleus meanwhile becomes slightly indented in the median line (Pl. 12, fig. 46) and then gradually constricts into two, the halves migrating to the periphery (Pl. 12, fig. 47). During this nuclear division the daughter-flagella execute very vigorous lashing movements, and a constriction appears at the flagellar end of the parent organism. A split appears at this end (Pl. 12, figs. 47–49), and, at the same time, vacuoles in the clear median area fuse, and thus the extension of the split is facilitated. The daughter-organisms rapidly separate from one another, their appearance at times being suggestive of diverging curved calipers (Pl. 12, figs. 51, 52). At length the two are practically in a straight line (Pl. 12, figs. 53–55), in which condition they remain for a short time and then finally separate.

The division of the pre-flagellate forms is initiated by the division of the blepharoplast, and is followed by the division of the nucleus and the appearance of vacuoles. A slight split appears at one end (Pl. 12, fig. 3), and the organism remains in this condition until the flagellum of each half has partly grown, when final separation is effected by their movements.

Sometimes repeated division of a pre-flagellate form occurs

and a rosette (Pl. 12, fig. 4) is produced, but the rapidity of the process of formation of flagella causes short duration of the rosette stage. On the other hand, repeated longitudinal division of flagellated individuals occurs, and as the individuals so produced do not separate immediately, rosettes (Pl. 12, fig. 56) are formed. In division, the posterior ends of the daughter-organisms are the last parts to separate. As the daughter-forms remain in proximity and themselves proceed to divide with rapidity, true division-rosettes are formed, in which the posterior ends of the organisms are central, while the flagella radiate out from the common centre. Such division-rosettes (Pl. 12, fig. 56) differ from the aggregation-rosettes (Pl. 12, figs. 41, 43; Pl. 13, figs. 95, 96) where the organisms become attached by their flagella. The distinction between the two forms of rosettes has not been shown by previous workers on C. melophagia.

Longitudinal division results in the formation of both equal and sub-equal daughter forms.

While the occurrence of equal longitudinal fission is the commoner (Pl. 12, figs. 50, 54, 55), I have seen cases of marked inequality in the size of the daughter-parasites, the one being very thin and narrow, the other considerably broader and thicker (Pl. 12, figs. 51, 53). As the entire process of sub-equal division has been watched in living organisms, there is no possibility of it being mistaken for anything else. The polymorphism resultant on division is strongly against the idea that there are sexual forms of Crithidia, and I have never seen the slightest indication that there is sexual dimorphism, in C. melophagia, C. gerridis, Herpetomonas jaculum, H. muscæ domesticæ, H. culicis, and a new Herpetomonas from Vespa crabro, all of which I have examined in the living condition (see Porter [1909] on C. gerridis and H. jaculum).

Division, usually twice repeated, is found to occur in parasites destined to encyst, and the resultant forms are very small. The first division is of the usual flagellate type (Pl. 13, fig. 97). The process of the second division rather

resembles that of the pre-flagellate stages, for before it is accomplished the flagella have almost disappeared. Sometimes no flagellum is visible at all, and the parasites look like dividing cysts.

On rare occasions the posterior end of a flagellate has divided before the anterior end (Pl. 13, fig. 98).

The Hereditary Infection of Melophagus ovinus by Crithidia melophagia.

Casual infection of Melophagus ovinus by the ingestion of post-flagellate cysts of Crithidia melophagia is fairly easily observed. The development of the parasite in the egg can only be studied with difficulty. I now wish to give a fuller account than exists up to the present of the processes leading up to the birth of Melophagus infected with Crithidia melophagia.

The first point to be determined was the way in which the Crithidia reached the egg. Infected Melophagus were carefully dissected so that no rupture of the gut was made. The ovaries also were dissected out and kept as far as possible in the position beside the gut that they occupied in life. Crithidia could be seen through the gut-wall moving actively about. Suddenly they concentrated in one place and soon began to pass through the wall, their posterior (blunt) end first. They rapidly swam direct to the ovaries and penetrated them in the same way, that is, with the non-flagellar end first. The flagellum was very rarely used as a boring organ to allow of the passage of the organism.

Penetration of the ovaries of their host by the parasites occurs in other cases, e. g. C. gerridis, H. jaculum, but the ova are apparently unattacked and the flagellates simply degenerate. But in the case of C. melophagia the organisms (Pl. 13, figs. 57, 59) make their way rapidly to the ova, to which they cling, whether the ova are mature or immature. In some cases one Crithidia only enters the egg (Pl. 13, fig. 58); at other times several penetrate it at once. In

penetration the blunt end of the flagellate enters the egg first. Occasionally the flagella are cast off as the Crithidia pass into the egg and remain on the outside.

In the case of older ova, the parasites seem to penetrate the egg at a definite spot (Pl. 13, fig. 58), which probably becomes the mouth of the embryo. Parasites invading older embryos enter by the embryonic mouth. Like Swingle I did not find parasites in the milk-glands or milk of Melophagus.

In investigations of the stages of C. melophagia in the egg and puparia I found that smear preparations were preferable to sections. Greater rapidity of manipulation and thinner preparations could be obtained by this means.

The method adopted was to prick the egg or open the young puparium and express the contents on to a slide. The contents were at once fixed and then were allowed to flow over the slide, so that no artificial spreading was required, and therefore no mechanical distortion or tearing of the parasites could occur. The preparations so made contained much fatty matter. The slides were treated with ether to remove the fat, and then after washing with absolute alcohol were stained and mounted in the usual manner.

Once within the egg the parasite gradually loses its flagellum (Pl. 13, figs. 61–63). This may be cast off entire, for flagella are found floating freely in the vitellus of eggs that had been treated with the utmost care in the manner previously detailed. In many cases the flagellum appears to be gradually absorbed (Pl. 13, figs. 64, 66). Longitudinal division of the flagellates in the egg may occur, though rarely.

The protoplasm of the Crithidia then concentrates round the nucleus and blepharoplast (Pl. 13, figs. 64–69) and the parasite gradually becomes more or less rounded (Pl. 13, figs. 70–73). Multiple division of both nucleus and blepharoplast then occurs (Pl. 13, figs. 74–77), and the daughter-blepharoplasts appear to pass outwards towards the periphery (Pl. 13,

figs. 76, 77). A "plasmodial"[1] form (Pl. 13, figs. 75, 77) is thus assumed. The protoplasm collects around the nuclei, and gradually fragmentation of the "plasmodium" occurs, the result being the formation of a number of small bodies, which rapidly round off, forming definite resting bodies (Pl. 13, figs. 78–81). Sometimes these resting bodies remain in proximity to one another, so forming groups (Pl. 13, figs. 80, 81). The parasites now measure only 1·5 μ to 4 μ long and 1 μ to 2·5 μ broad. Sometimes one chromatic mass (Pl. 13, fig. 82) only can be distinguished. Often both nucleus and blepharoplast (Pl. 13, figs. 80, 81, 83, 84) are present.

As the embryo grows the rounded forms of the parasite in the stomach (which is the chief cavity within the young Melophagus) also grow (Pl. 13, figs. 82–84). The Crithidia then undergo multiple division, small rosettes (Pl. 13, figs. 85–88), analogous to pre-flagellate rosettes, being produced. The division clusters may separate, giving rise to small, pear-shaped or ovoid individuals (Pl. 13, figs. 89–94), or they may remain as a rosette (Pl. 13, fig. 88) for some time. Whether the Crithidia remain as groups or become isolated as oval non-flagellated bodies, they undergo no further development for a considerable period. In fact, when the young Melophagus is hatched, a month after extrusion of the puparium, there is still no further differentiation in the parasite.

Freshly hatched Melophagus do not contain the fully developed flagellates, but the rounded or pear-shaped pre-flagellate forms (Pl. 13, figs. 92–94) and rosettes (Pl. 13, fig. 88) may be present. The parasites appear to lie dormant for a day or two, during which time the young insect does not appear to suck blood. Soon after the first meal of blood is taken, rapid development of the pre-flagellate forms occurs, and the adult flagellate form of the Crithidia is quickly assumed.

[1] A plasmodium is really a multinucleate mass of protoplasm formed by fusion of small amœbæ. However, the term is sometimes used, as in describing certain Haplosporidia, for a multinucleate mass of protoplasm formed by division.

Casual Infection.

The method of cross-infection in many species of Crithidia has not been demonstrated, but in the cases known the casual or contaminative method seems to prevail. The post-flagellate stages of Crithidia gerridis and C. tabani are known, and the cysts of these parasites are shed in the fæces of the insectan hosts. The crithidian cysts are swallowed by new hosts when they feed on material accidentally contaminated by the fæces of their neighbours. The cysts then develop in the alimentary tracts of the new hosts. Melophagus ovinus also becomes infected with its Crithidia by the casual method.

When studying C. melophagia I have noticed that the fæces of Melophagus ovinus are voided near spots on the sheep from which blood has recently been sucked (particularly is this the case at times of extrusion of puparia); that the fæces contain crithidian, post-flagellate cysts, and sometimes active flagellates; and that other Melophagus, feeding at the same spot, have thrust their proboscides into the semi-fluid fæces to reach the blood of the sheep. Ingestion of cysts under such circumstances is easy. The ingestion of fæces has been seen particularly well when a number of keds have been kept confined to a small area of the sheep's body.

At shearing a slight injury was caused to one sheep, and the keds seemed to collect round the small bleeding patch. Their habits were carefully observed then, and were similar to those described above. I do not agree with Swingle that casual infection is only a remote possibility; to my mind it is a certainty.

A modified contaminative cross-infection is rendered possible by the cannibalistic habit of Melophagus ovinus. The keds have been seen to attack one another, the point of seizure invariably being at the end of the abdomen near the anus. When a ked so attacked has been freed from its aggressor and then dissected, I have found that the abdominal cavity was almost empty, the viscera having been devoured

by the attacking ked. By this cannibalistic habit it is possible for Melophagus ovinus to acquire practically every stage of Crithidia melophagia direct, and this is probably a subsidiary method of spreading the parasite.

Environmental Effects.

Crithidia melophagia shows less response to slight changes of environment than does C. gerridis or Herpetomonas jaculum, both of which I have studied. Nevertheless, under certain conditions remarkable effects have been produced by relatively simple means, and these may now be recorded.

(1) Response to light.—Increased intensity of white light produces increased velocity of movement of Crithidia melophagia.

Green light somewhat retards the movements of the organism. This is also the case with Herpetomonas jaculum.

Intense light causes aggregation-rosettes of C. melophagia to separate.

C. melophagia lives very much longer in diffuse light than in bright light.

(2) Response to changes of temperature.—C. melophagia can live at a temperature just below that of the blood of the sheep, but the flagellates are killed at a temperature above 40° C.

At room temperature (15° C.) the parasites will live for several hours.

(3) Response to change of medium.—Though the flagellates normally live surrounded by fluid blood (a discussion of which will be given in Appendix III), yet they can live in other media and can resist the effects of such media to varying degrees.

(*a*) Tap-water when added to the parasites in the gut-liquid seemed to have little effect. Though the movements of the flagellate became slightly more active, this was possibly

due to the greater space in which the parasites could move, the débris being distributed over a greater area than before.

(*b*) 0·75 per cent. NaCl solution increased the activity of the parasites.

Five parts of tap-water added to one part of 0·75 per cent. NaCl solution containing Crithidia caused the flagellates to move more rapidly, the spiral boring movements of the flagellum becoming more exaggerated.

(*c*) Caustic potash.—Two per cent. solution killed all the Crithidia within a minute; 1 per cent. potash solution killed them in from seven to twelve minutes, but their bodies were not dissolved, this pointing to the chitinoid nature of the thin periplast or ectoplasm.

(*d*) Acetic acid.—One third per cent. aqueous solution had the effect of swelling the parasites, which then died.

(*e*) Grape-sugar.—A most remarkable effect was that produced on C. melophagia by a solution containing a very small amount of grape-sugar. When this was added to the parasites they commenced to divide very rapidly, and many divisions occurred. To ascertain if there were a connection between this division and the occurrence of sugar in the natural medium of the parasites, some experiments were made. The results were as follows:

(i) Sheep-serum contains a very small amount of grape-sugar.

(ii) The liquid obtained when wool cut from the sheep was boiled with water and then concentrated also showed traces of sugar. There were, then, these two sources from which the ked probably could obtain minute quantities of sugar. It is possible that the traces of sugar may take a small share in stimulating division of C. melophagia, which goes on more rapidly in the stomach of the ked than elsewhere.

(*f*) Fresh blood (human or sheep's) added to a preparation of living Crithidia caused the parasites to move away to areas where the blood was somewhat less concentrated, where they proceeded to divide rapidly.

(*g*) Dilute glycerine killed C. melophagia almost at once. Vaseline had the same effect after a very short time.

(4) Effect of a parasitic fungus of Melophagus ovinus on C. melophagia.—The presence of a fungus in Melophagus ovinus has already been mentioned. As I very rarely found the fungus and Crithidia co-existing in a ked, it was deemed advisable to find out any possible interrelation of the two parasites. The Malpighian tubules of the ked—often blocked with fungus—were the most heavily infected organs. Fungus taken from the Malpighian tubes was crushed with a little water. The emulsion, which probably contained an enzyme, was added to a preparation of actively moving C. melophagia. The movements of the flagellates slowed at once, their protoplasm became much more vacuolated, and the parasites appeared to burst. After seven to nine minutes no living Crithidia were to be seen.

The fungus-infected Melophagus ovinus seems widely distributed. Specimens from Scotland were practically always heavily infected with it, and some keds from each locality tried in England also were infected. These keds very rarely contained Crithidia. The fungus seems to have a pathogenic action upon the flagellate, and I believe that the co-existence of the fungus and Crithidia for long together is almost impossible.

General Remarks.

Regarding the previous work done on the genus Crithidia, I have already noted most of the memoirs dealing with the subject in my paper on Crithidia gerridis (1909). Consequently the remarks now appended relate especially to the flagellate of Melophagus ovinus.

E. Pfeiffer (1905) first briefly described a flagellate as occurring in the gut of Melophagus ovinus. He mentions that L. Pfeiffer had seen and recorded the parasite in 1895. The flagellate stage only was described, and no definite name was given to the organism, which was stated to be "like a trypanosome."

P. C. Flu (1908) published an account of the flagellate under the name of Crithidia melophagia. Flu stated that he saw parasites in the ovary of Melophagus, and small forms in the larva, but was unable to determine the mode of infection of the host.

L. D. Swingle (1909), working in Nebraska, wrote a description of the parasite, calling it C. melophagi. From a private communication I learn that Swingle's work was completed, but not published, before Flu's paper appeared, thus accounting for the specific name melophagi (described as new), which cannot stand. The chief value of Swingle's work lies in the fact that he described rounded and "plasmodial" stages of the parasite as occurring in the egg of the host.

While Swingle was working in Nebraska, I was investigating the parasite independently in England. It gives me great pleasure to be able to confirm Swingle's work, and to add many more details concerning the modes of infection of the parasite and its general life-history.

Summary.

(1) Crithidia melophagia is a flagellate occurring in the alimentary tract, ovaries, ova, and puparia of Melophagus ovinus.

(2) The parasite has three stages in its existence, a pre-flagellate stage (Pl. 12, figs. 1–20), passed chiefly in the crop and fore-gut of the insect host, a flagellate stage (Pl. 12, figs. 21–44), occurring chiefly in the posterior two thirds of the gut, and a post-flagellate stage, occurring either in the rectum and fæces (Pl. 13, figs. 97–114) or in the ova and pupæ (Pl. 13, figs. 57–94).

(3) The pre-flagellate stage is passed through very rapidly. These parasites are small, usually oval bodies, 1μ to 4·5 μ by 4·5 μ to 6 μ, with round nuclei and bar-like blepharoplasts. The flagellum arises near the blepharoplast from a chromatophile area. Division of pre-flagellates may occur (Pl. 12, fig. 4).

(4) The flagellate forms are from 12 μ to 75 μ long, and 1·5 μ to 2·8 μ broad (including the flagellum). The general protoplasm is slightly alveolar. The nucleus is vesicular. The blepharoplast is well marked, rod-like, usually anterior to the nucleus, and generally homogeneous.

Chromidia may occur as isolated granules.

(5) The undulating membrane and flagellum are well marked. There are indications of myonemes (Pl. 12, figs. 40, 45) in some stained specimens, but the myonemes are more evident in some living specimens. The membrane is of great use in securing smoothness of motion. The flagellum is long and forms a chromatic edge to the membrane. A basal granule may occur near the root of the flagellum.

(6) The post-flagellate stage in the host's rectum (Pl. 13, figs. 97–114) gives rise to resistant (resting) bodies that are passed out as cysts with the fæces and serve to infect new hosts. The cysts measure, on the average, 4 μ by 2·5 μ. The flagellates divide, usually twice, and the four small forms thus produced lose their flagella, become round, and then invested with a thin gelatinous wall.

(7) The post-flagellate stages in the ova and puparia of Melophagus (Pl. 13, figs. 57–94) serve for the hereditary transmission of C. melophagia. The flagellates pass through the wall of the gut near the anterior ends of the ovaries, swarm towards and enter the ovaries and penetrate the ova—the posterior (aflagellar) end of the parasite being used in penetration. Within the ova each parasite loses its flagellum and becomes ovoid or rounded (Pl. 13, figs. 64–73). Nuclear multiplication follows and "plasmodial" forms are produced (Pl. 13, figs. 74–77). These give rise to small, rounded bodies (Pl. 13, figs. 83, 84) about 3 μ by 2 μ which undergo multiple fission to form rosettes (Pl. 13, fig. 88), which give rise to the typical pre-flagellates.

(8) The young Melophagus do not show flagellates until after their first feed of blood, the blood stimulating the pre-flagellates to form flagella.

(9) Multiplication of C. melophagia by longitudinal

division occurs in both the pre-flagellate and the flagellate stages of the parasite.

(10) Infection of Melophagus ovinus with C. melophagia is either hereditary or casual. In the case of casual infection the insects ingest the post-flagellates voided with the fæces of other Melophagus.

(11) A very dilute solution of grape-sugar causes the parasites to divide. There are only traces of sugar in both sheep-serum and wool extract.

(12) Sheep's blood or human blood added to the Crithidia also increased the rapidity of their growth and division.

(13) A fungus present in the Malpighian tubules and gut of the ked (see Appendix II) has a rapid, fatal effect on the Crithidia.

(14) An anti-coagulin is present in the salivary glands, stomach and intestine of Melophagus ovinus (see Appendix III).

(15) A new spirochæte, S. melophagi, was found in the gut, ovaries and puparia of a few of the Melophagus examined (see Appendix I).

Appendix I.

On the Occurrence of a Spirochæte, S. melophagi, n. sp., in Melophagus ovinus.

I wish to record the occurrence of a rare spirochæte in the gut, ovaries and puparia of Melophagus ovinus. The spirochæte was observed in life in the above-mentioned organs of a very few of the Melophagus examined, and at very different periods of the year (February, September, October). Very few spirochætes occurred, and consequently it is impossible to give full details regarding size and structure. The organisms seen were from 10 μ to 30 μ long and were narrow. They vary in length, some being practically half the length of others, indicating the probability of transverse division. As some parasites were thicker than others there

is the inference that longitudinal division takes place. This would be in accordance with the behaviour of other spirochætes, for Fantham (1907–8–9) has shown that both forms of division occur in S. balbianii and S. anodontæ. I (1909) also have observed the same, while the joint researches of Fantham and myself (1909) have demonstrated that both directions of division occur in S. recurrentis and S. duttoni, and that there is a periodicity in the direction of division.

The movements of S. melophagi are fairly active, and are of the typical spirochæte nature, namely, of forward progression accompanied by spiral or corkscrew rotation on its course.

The occurrence of S. melophagi in the ovaries, ova and puparia of the ked is of much interest, for it indicates that the spirochæte is transmitted hereditarily. Hence Melophagus ovinus can transmit both Crithidia melophagia and Spirochæta melophagi to its offspring.

Appendix II.

Note on a Fungus found in the Malpighian Tubules and Intestine of Melophagus ovinus.

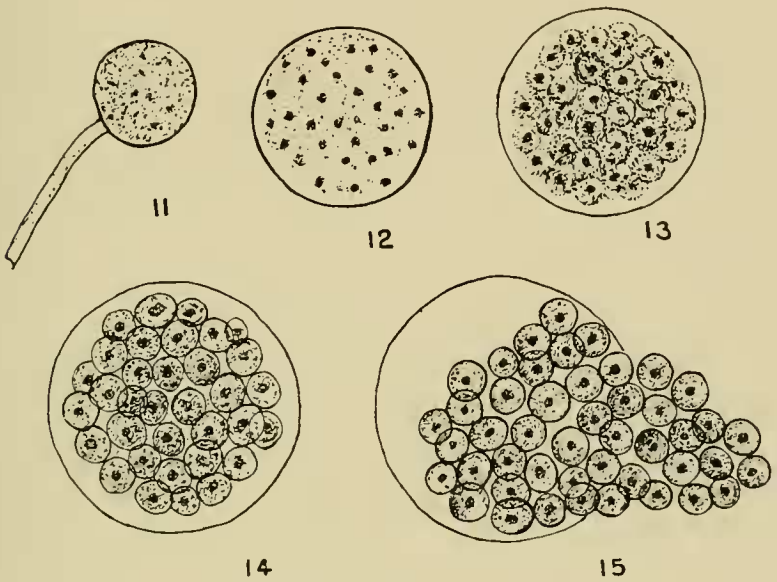
A fungus was present in many specimens of Melophagus ovinus examined, especially those obtained from Scotland. Crithidia were not seen in the "keds" received from Scotland, and I have shown experimentally that the action of the fungus is fatal to the flagellate.

The fungus occurred chiefly in the Malpighian tubules of the insect, and to a lesser extent in the intestine. The Malpighian tubules were frequently blocked by the fungus. A brief description of the fungus may now be given.

The hyphæ were long and filamentous with few septa. Many spores were produced. At the extremity of some hyphæ globular heads were formed, possibly due to sexual processes. The globular bodies contained many nuclei (text-fig. 11) fairly evenly distributed through the protoplasm.

Nuclei and protoplasm then shrank away from the wall of the rounded body—provisionally called a sporangium (text-fig. 12)—so that a space intervened. Segregation of the protoplasm round the nuclei followed (text-fig. 13), and a morula-like body resulted. The morula differentiated into a mass of rounded spores (text-fig. 14), each of which formed a spore coat for itself. The sporangium ultimately ruptured

TEXT-FIGURES 11–15.



Fungus parasitic in Melophagus ovinus.

Text-fig. 11.—Hypha with globular head.
Text-fig. 12.—Differentiation of nuclei within the head (sporangium).
Text-fig. 13.—Spores forming in sporangium.
Text-fig. 14.—Mature sporangium.
Text-fig. 15.—Dehiscing sporangium.

(text-fig. 15), and the numerous small spores were set free. Some spores remained in the Malpighian tubes, others passed out into the intestine and were voided with the fæces.

Parasitic fungi have been previously recorded in insects, for example, in the house-fly, caterpillar, mosquito. The fungus mentioned by Schaudinn in Culex was probably a member of the Entomophthoreæ, or related thereto. The

fungus infesting Melophagus ovinus seems to be more nearly allied to the Peronosporeæ.

I learn from a private communication that a similar fungus was found last year by Dr. H. B. Fantham, of Cambridge, in the alimentary tract and Malpighian tubes of the grouse-fly, Ornithomyia lagopodis. From examination of a preparation of the fungus of Ornithomyia, kindly lent to me, I believe that the fungi of the grouse-fly and the sheep-ked are very closely related.

Appendix III.

On the Occurrence of an Anti-coagulin in the Alimentary Canal of Melophagus ovinus, and its Significance in Relation to Crithidia melophagia.

The pronounced and peculiar brightness of the blood in the crop and fore-part of the stomach of the keds examined was noticed very early in the investigation. The blood of the sheep in the stomach of keds that had not fed for as long as three days was still practically fluid and had not coagulated much, while twelve to twenty-four hours after feeding the blood had not coagulated at all. This led me to suspect that an anti-coagulin, such as had been described in a tick (Argas persicus) by Nuttall and Strickland (1908), was present here also, and a series of tests were performed at different times which verified this inference. Every test that I performed had the same result—coagulation was delayed.

The method of testing was simple. Separate emulsions of the salivary glands, stomach, and intestine of Melophagus ovinus were made with 0·75 per cent. NaCl solution. A known quantity—about 0·5 c.c. of human blood from a pricked finger—was then mixed with the same quantity of organ-emulsion, while for control purposes the same quantity of blood mixed with 0·75 per cent. NaCl solution was used. The test fluid and the control fluid were taken up in small glass capillaries, and the test was applied by blowing out the

liquid at stated times and noting when coagulation occurred in each. Typical results of these experiments are tabulated below:

A. Adult Melophagus.

Experiment.	Coagulation period of blood and organ-emulsion.	Coagulation period of blood and ·75 NaCl solution.
(1) Salivary gland	20 min.	7–8 min.
(2) ,, ,,	22 ,,	8 ,,
(3) Intestine	14 ,,	8 ,,
(4) ,,	14 ,, 30 sec.	8 ,,

Obviously an anti-coagulin was present, for considerable delay of clotting occurred.

B. Young Melophagus.—Here the interval between the clotting of the test and control preparations was noted. A few typical results are given:

(1) Blood mixed with emulsion of the salivary glands clotted nine minutes after the control.

(2) Emulsions of intestine added to blood caused the latter to take three times as long to clot as the control preparations took.

Comparing the behaviour of the emulsions of the salivary glands of young and of older keds, the anti-coagulin seems to be more strongly developed in the salivary glands of the older keds, while a similar comparison between the intestinal emulsions would tend to show that the anti-coagulin was more abundant in the intestines of young keds.

The temperature at which the anti-coagulin was destroyed was also investigated. It was found that below 50° C. the anti-coagulin would act. At about 55° C. its action was checked. When 60° C. was reached it was destroyed.

Human blood mixed with emulsions of any part of the alimentary canal at once assumed the vivid red hue so noticeable in the blood removed from the gut of the keds.

The red blood-corpuscles of the sheep, seen en masse, appear far brighter on adding emulsions of the gut of the ked containing the anti-coagulin. When much water was added to normal blood, hæmolysis occurred, and the colour

of the solution so obtained was made much brighter when an emulsion of crushed salivary glands of the ked was added to it. The leucocytes of the sheep's blood occurring in the gut of the ked do not appear to be affected in any way by the anti-coagulin.

Anti-coagulin appears to be found in all parts of the alimentary canal of the ked and to decrease in amount from before backwards. As before mentioned, I determined experimentally that freshly shed, and therefore fluid, blood acted as a stimulant to division of the Crithidia. This artificial condition is the counterpart of the natural condition of the blood within the fore-gut of the ked. There, owing to the action of the anti-coagulin, the freshly ingested sheep's blood does not clot, but remains fluid. It is probable that Crithidia within the gut are stimulated by this fluid blood, and divide rapidly. I obtained similar results in the case of Herpetomonas jaculum, where "division of the flagellate Herpetomonad takes place rapidly under natural conditions after ingestion of blood by the host" (Porter [1909], p. 382). If the Crithidia are in the pre-flagellate condition the rapid multiplication is followed by the outgrowth of flagella, after which the organisms separate and pass further down the alimentary canal. The presence of anti-coagulin, from the salivary glands, in the contents of the fore-gut of the ked may be the cause of the rapidity with which the pre-flagellate stage of Crithidia melophagia is passed through, the blood, kept fluid by the anti-coagulin, acting as a stimulus to further development.

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EXPLANATION OF PLATES 12 AND 13,

Illustrating Miss Annie Porter's paper on "Crithidia melophagia."

[All figures were outlined with an Abbé-Zeiss camera-lucida, using a 2 mm. apochromatic (Zeiss), or $\frac{1}{12}$ inch achromatic (Zeiss) objective, and compensating oculars 8 and 12 of Zeiss. The magnification is in all cases approximately 1500 diameters, except where otherwise stated.]

PLATE 12.

Figs. 1–20.—Pre-flagellate Stages.

Fig. 1.—Pre-flagellate with round nucleus, bar-like blepharoplast. No flagellum. Crop. Giemsa.

Fig. 2.—Oval pre-flagellate. Blepharoplast slightly constricted. Crop. Delafield's hæmatoxylin.

Fig. 3.—Dividing pre-flagellate. Crop. Delafield's hæmatoxylin.

Fig. 4.—Division rosette of pre-flagellates. Two individuals again dividing. Crop. Delafield's hæmatoxylin.

Figs. 5–8.—Elongating pre-flagellates. Crop. Thionin.

Fig. 9.—Large preflagellate, with round nucleus, rod-like blepharoplast, flagellum just differentiating. Crop. Giemsa.

Fig. 10.—Rounded form. Flagellum longer than in fig. 9. Crop. Giemsa.

Fig. 11.—Smaller parasite with large nucleus and long flagellum. Crop. Delafield's hæmatoxylin.

Fig. 12.—Parasite, showing elongation of flagellar (anterior) end of the body. Crop. Giemsa.

Figs. 13 and 14.—Crithidia with elongated posterior ends. Anterior part of stomach. Giemsa.

Fig. 15.—Pre-flagellate with posterior blepharoplast. Crop. Giemsa.

Fig. 16.—Parasite with anterior end more developed. Crop. Giemsa.

Figs. 17 and 18.—Almost mature flagellates, membranes showing myonemes. Crop. Giesma.

Figs. 19 and 20.—Practically adult flagellates. Fore-part of stomach. Thionin.

Figs. 21–43.—Flagellate Stage.

Fig. 21.—Small flagellate. Nucleus with chromatin in granules extending part way across the body. Rod-like blepharoplast. Intestine. Giemsa.

Fig. 22.—Flagellate, with well-marked myonemes on the body. Stomach. Gentian violet. × 2250 approximately.

Fig. 23.—Parasite, with flagellum almost continuous with the blepharoplast. Nucleus with central chromatin. Stomach. Delafield's hæmatoxylin.

Fig. 24.—*Crithidia* showing blepharoplast posterior to the nucleus —an uncommon condition. Stomach. Giemsa.

Figs. 25, 26.—Flagellates showing chromidia in their posterior ends. Chromatin of nucleus in bars. Stomach. Giemsa.

Figs. 27–29.—Parasites with somewhat pointed posterior ends. Chromidia present in fig. 29. Intestine. Thionin.

Figs. 30, 31.—*Crithidia* showing somewhat alveolar protoplasm. Stomach. Thionin. × 2250 approximately.

Fig. 32.—Flagellate with blunt posterior end, round nucleus containing large chromatin granules, and extending across complete breadth of body; blepharoplast curved. Stomach. Thionin. × 2250 approximately.

Fig. 33.—Parasite with scattered chromidia. Blepharoplast slightly posterior to and to the side of the nucleus. End of crop. Giemsa.

Fig. 34.—Crithidia with large oval blepharoplast. Stomach. Giemsa.

Fig. 35.—Narrow parasite. Intestine. Giemsa.

Figs. 36, 37.—Longer parasites with many chromidia. Stomach. Iron-hæmatoxylin.

Fig. 38.—Flagellate showing alveolar protoplasm, nucleus and blepharoplast almost in contact. Intestine. Thionin. × 2250 approximately.

Fig. 39.—Long form. Nucleus with chromatin arranged in bars. Oval blepharoplast. Membrane distinct. Intestine. Giemsa.

Fig. 40.—Long parasite with thick flagellum. Myonemes present on body. Blepharoplast showing constriction, so about to divide. Chromatin of nucleus in large masses. Stomach. Delafield's hæmatoxylin.

Fig. 41.—Small aggregation-rosette, showing entanglement of large and small flagellates. Stomach. Giemsa.

Fig. 42.—Flagellate with rounded nucleus and posterior blepharoplast. Basal granule near root of flagellum. Myonemes in membrane. Intestine. Iron-hæmatoxylin.

Fig. 43.—Large rosette. Many parasites shown aggregated around a piece of débris. The flagella serve as points of attachment, therein differing from a division-rosette. Common in stomach and intestine. Delafield's hæmatoxylin.

Figs. 44–56.—Stages in Division.

Fig. 44.—Parasite showing constricted blepharoplast with clear area around it. Chromatin in nucleus arranged in masses at periphery. Intestine. Thionin. × 2250 approximately.

Fig. 45.—Stage similar to fig. 44. Well-marked myonemes on body and membrane. Giemsa. × 2250 approximately.

Fig. 46.—Parasite with both nucleus and blepharoplast constricted. Flagellum beginning to split at base. Stomach. Delafield's hæmatoxylin.

Fig. 47.—Flagellate with anterior end of body, nucleus and blepharoplast all divided. Stomach. Delafield's hæmatoxylin.

Figs. 48, 49.—Somewhat rounded parasites; bodies of daughter-forms not yet diverging from one another. Stomach. Thionin.

Fig. 50.—Daughter-organisms forming a V. Stomach. Giemsa.

Figs. 51, 52.—Further stages in the divergence of the bodies of the

daughter-forms. The flagella have interlocked. Intestine. Delafield's hæmatoxylin. The parasites represented in fig. 51 divided sub-equally.

Fig. 53.—Sub-equal division. Daughter-organisms are almost separated. Intestine. Delafield's hæmatoxylin.

Figs. 54, 55.—Parasites about to separate. Stomach. Giemsa.

Fig. 56.—True division-rosette. The separation of the daughter-individuals takes place from the flagellar end backwards, so that in a rosette the posterior ends of the organisms are centrally directed. Stomach. Thionin.

PLATE 13.

Figs. 57–94.—Stages of the Parasite in the Ovary, Eggs, and Puparia.

(The eggs in figs. 58, 64, 65 are represented diagrammatically.)

Fig. 57.—The flagellate as it penetrates the ovary. Delafield's hæmatoxylin.

Fig. 58.—Flagellate in the act of penetrating a young egg, the blunt end of the parasite being used. Thionin. The egg of Melophagus ovinus is represented diagrammatically.

Figs. 59, 60.—Flagellates from ovary. Flagella somewhat reduced. Giemsa.

Figs. 61–63.—Flagellates from within the egg. Giemsa.

Figs. 64, 65.—Rounding-up forms of C. melophagia within eggs. Delafield's hæmatoxylin and fresh preparations. Eggs of Melophagus represented diagrammatically.

Figs. 66–72.—Series of parasites showing successive stages in shortening and rounding-up of flagellates when within the eggs. Delafield's hæmatoxylin.

Figs. 73, 74.—Parasites showing nuclear division. Very young puparium. Giemsa.

Figs. 75–77.—"Plasmodial" stages of C. melophagia in developing puparia. Peripheral blepharoplasts seen. Giemsa and fresh preparations.

Figs. 78–81.—Rounded parasites resulting from plasmodial forms. Delafield's hæmatoxylin.

Figs. 82–84.—Parasites produced by growth of forms similar to those shown in fig. 81. Giemsa.

Figs. 85–87.—Rosettes of somewhat oval parasites from young puparium. Delafield's hæmatoxylin.

Fig. 88. — Well-defined division-rosette from mature puparium. Giemsa.

Figs. 89–91.—Dividing forms. Mature puparium. Giemsa.

Figs. 92–94.—Parasites resembling pre-flagellates produced from cyst. Mature puparium. Delafield's hæmatoxylin.

Figs. 95, 96.—Small aggregation-rosettes. Intestine. Thionin.

Figs. 97–114.—Post-flagellate Stages in Rectum.

Fig. 97.—Parasite dividing prior to encystment. Intestine. Thionin.

Fig. 98.—Uncommon form of division, occasionally seen in living specimens. Rectum. Giemsa.

Fig. 99.—Small form. Flagellum in process of absorption. Rectum. Giemsa.

Fig. 100.—Parasite showing concentration of protoplasm in the region of the nucleus. Rectum. Giemsa.

Fig. 101.—Form common in rectum. Body much flattened. Flagellum disappearing. Delafield's hæmatoxylin.

Figs. 102–108.—Parasites showing progressive disappearance of flagellum. Rectum. Thionin.

Figs. 109–112.—Post-flagellate cysts from rectum. Giemsa.

Fig. 113.—Post-flagellate cyst from fæces of *Melophagus ovinus*. Giemsa.

Fig. 114.—Thick-walled cyst. Rectum. Giemsa.